

Attorney Docket No.: 44158/244344 (SJ-0029)  
Inventors: Schuetz et al.  
Serial No.: 09/974,619  
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**Amendments to the Specification:**

Please replace the paragraph beginning at page 18, line 20, with the following:

In particular, a polymorphism is identified in intron 3 of the CYP3A5 gene at the position corresponding to nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of TAAAGAGCTCTTTTGTCTTTCAGTAT (SEQ ID NO:73) ~~of Figure 3~~) and in exon 7 of the CYP3A5 gene at the position corresponding to nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of CACAAGACCCCTTTGTGGAGAGCACTAAGAAG (SEQ ID NO:74) ~~of Figure 5~~).

Please replace the paragraph beginning at page 18, line 25, with the following:

A sequence variation in the CYP3A5 gene wherein an Adenine (A) in intron 3 is altered to a Guanine (G) at the position corresponding to nucleotide 22,893 GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~) is one of the polymorphisms identified herein. This polymorphism leads to the expression of a truncated CYP3A5 protein with little or no catalytic activity.

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Please replace the paragraph beginning at page 19, line 1, with the following:

The invention further provides a sequence variation in the CYP3A5 gene wherein a Guanine (G) in exon 7 at the position corresponding to nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) is altered to an Adenine (A) in this same position. This polymorphism leads to expression of an altered CYP3A5 with low catalytic activity that is missing most of the amino acids encoded by exon 7.

Please replace the paragraph beginning at page 19, line 7 with the following:

Accordingly to the present invention, the presence of either the A to G polymorphism at the position corresponding to nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~) or the G to A polymorphism at the position corresponding to nucleotide 30,597 of GENBANK sequence accession No. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) in the CYP3A5 gene is predictive of reduced CYP3A5 expression.

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Please replace the paragraph beginning at page 30, line 18 with the following:

Assays and methods have been developed and are provided and described herein for determination of the CYP3A5 sequence, particularly of the intron 3 sequence, and most particularly of the nucleotide 22,893 (relative to GENBANK AC005020; also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~) genotype of an individual. Assays and methods have been developed and are provided and described herein for determination of the CYP3A5 sequence, particularly of the exon 7 sequence, and most particularly of the nucleotide 30,597 (relative to GENBANK AC005020; also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) genotype of an individual.

Please replace the paragraph beginning at page 32, line 11 with the following:

The step (b) may be performed utilizing any method of amplification, including polymerase chain reaction (PCR), ligase chain reaction (Barany, F. (1991) Proc. Natl. Acad. Sci. 88:189-193), rolling circle amplification (Lizardi, P.M. et al. (1998) Nature Genetics 19:225-232), strand displacement amplification (Walker, G.T. et al. (1992) Proc. Natl. Acad. Sci. 89:392-396) or

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alternatively any means or method whereby concentration or sequestration of sufficient amounts of the cytochrome P450 3A5 (CYP3A5) nucleic acid for analysis may be obtained. The primers for use in amplification of at least the intron region and/or the exon 7 region of CYP3A5 may be selected and utilized by the skilled artisan employing the sequence of cytochrome P450 3A5 (CYP3A5) as available at the National Center for Biotechnology Information (NCBI) ncbi with the extension nlm.nih.gov of the world wide web as GENBANK entry AC005020, portions of which are depicted in ~~sequences of Figures 3 and 5 as~~ SEQ ID NO:73 and 74, respectively, the complete sequence of *Homo sapiens* BAC clone Gs1-259H13 (Sulston, J.E. and Waterston, R. (1998) Genome Res. 8(11), 1097-1108). This particular sequence was utilized in the design and sequence of primers exemplified herein. In addition, GENBANK entry L26985 which sequence was published by Schuetz et al. (Schuetz, J. et al. (1995) Biochem Biophys Acta 1261:161-165). This sequence was originally described as a CYP3A5 pseudogene, but is actually a spliced variant mRNA, similar to the CYP3A5\*3 allele product described herein. Particular exemplary primers are provided herein and include oligonucleotide primers having the sequence set out in SEQ ID NOS: 16, 24-27 and 30-32. Based on the sequence of the mutant alleles provided herein, PCR primers are constructed that

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are complementary to the region of the mutant allele encompassing the point mutation. A primer consists of a consecutive sequence of polynucleotides complementary to any region in the allele encompassing the position which is mutated in the mutant allele. The size of these amplification/PCR primers range anywhere from five bases to hundreds of bases. However, the preferred size of a primer is in the range from 10 to 50 bases, most preferably from 15 to 35 bases. As the size of the primer decreases so does the specificity of the primer for the targeted region. Hence, even though a primer which is less than five bases long will bind to the targeted region, it also has an increased chance of binding to other regions of the template polynucleotide which are not in the targeted region and do not contain the polymorphic/mutated base. Conversely, a larger primer provides for greater specificity, however, it becomes quite cumbersome to make and manipulate a very large fragment. Nevertheless, when necessary, large fragments are employed in the method of the present invention. To amplify the region of the genomic DNA of the individual patient, primers to one or both sides of the targeted position, for instance the third intron (intron 3) and particularly the A/G point mutation at nucleotide 22,893 (relative to GENBANK AC005020; also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~), and also the

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position in exon 7 and particularly the G/A point mutation at nucleotide 30,597 (relative to GENBANK AC005020; also depicted herein as nucleotide 29 of SEQ ID NO: 74 ~~of Figure 5~~), are made and used in a PCR amplification reaction, using known methods in the art (e.g. Massachusetts General Hospital & Harvard Medical School, Current Protocols In Molecular Biology, Chapter 15 (Green Publishing Associates and Wiley-Interscience 1991) and as particularly exemplified herein.

Please replace the paragraph beginning at page 34, line 22 with the following:

Following amplification, the PCR product may be sequenced, subjected to a second round of amplification, or otherwise analyzed in step (c). The sequence may be determined using any of various methods known in the art, including but not limited to traditional sequencing methodologies and more rapid and high throughput mini-sequencing or pyrosequencing, including but not limited to those exemplified in Cai et al., Sun et al. and Ahmadian et al., which references are incorporated herein in their entirety by reference (Cai, H et al. (2000) Genomics 66 (2):135-143; Sun, X et al. (2000) Nucleic Acids Res 28(12):E68; Ahmadian, A. et al. (2000) Anal Biochem 280(1):103-110). In

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utilizing certain of these particularly sensitive and efficient sequencing methodologies it may, in fact, not be necessary to perform the (b) amplification step, provided that suitable starting nucleic acid is isolated in (a) for analysis. By utilizing methods which do not require sequencing and whereby the single nucleotide polymorphism, particularly the nucleotide 22,893 (relative to GENBANK AC005020; also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~) and nucleotide 30,597 (relative to GENBANK AC005020; also depicted as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) allelic sequences can be directly determined or inferred, one can rapidly screen genomic DNA from many individuals. A two step or two round amplification approach has been used successfully to examine polymorphisms in the CYP2D6 and in thiopurine methyltransferase (TPMT) genes (Evans et al., Pharmacogenetics, 1:143-148, 1991.). As exemplified herein, a nested PCR approach can be utilized. The first round of amplification is used to amplify the gene segment that may contain the mutation of interest. The second round of amplification can utilize one of the common primers as its first primer but importantly makes use of a second primer for either the wild-type sequence or for the mutation of interest, and using PCR conditions that produce sequence specific amplification.

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Alternatively, restriction enzyme digestion of the first or second round PCR product may be used to detect the presence or absence of a mutation, when a restriction site is either gained or lost. The second round of amplification may utilize an allele specific system, for instance, a mismatch directed primer, wherein a base change is specifically introduced by the primer, thereby generating a restriction site at or near the site of the point mutation in a particular allele. Further an allele specific system, for instance, a mismatch directed primer, may be used as one primer in a single round of amplification, wherein a base change is specifically introduced by the primer, thereby generating a restriction site at or near the site of the point mutation in a particular allele (this approach is exemplified herein in Example 3). Alternatively, an allele specific oligonucleotide, ligase chain reaction, etc. may be utilized so as to generate product only in the presence of a particular base or provide products which are distinguishable by dye, label, size, etc. in each case.

Please replace the paragraph beginning at page 36, line 1, with the following:

Other rapid pharmacogenetic single nucleotide polymorphism



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(SNP) screening technologies which can be employed and are contemplated as suitable for step (c) currently exist and could be utilized by the skilled artisan to identify or characterize the CYP3A5\*1, CYP3A5\*3 and CYP3A5\*6 alleles and particularly the nucleotide 22,893 (relative to GENBANK AC005020; also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~) and nucleotide 30,597 (relative to GENBANK AC005020; also depicted herein as nucleotide 29 of SEQ ID NO: 74 ~~of Figure 5~~) SNPs. Various detection methodologies are presently available or offered by commercial companies, including Aclara Biosciences, Orchid Biosciences, Qiagen Genomics, PPGX, and Affymetrix. Exemplary such SNP detection methodologies, particularly those of Orchid Biosciences, are provided in United States Patent Numbers 6,013,431, 6,004,744, 5,952,174 and 5,939,291, which are incorporated herein by reference in their entirety. Qiagen Genomics utilizes a Masscode system for SNP genotyping, whereby a mass spectrometer is utilized to image/detect Masscode tags attached to DNA molecules via a photochemical linker. A mass-tagged system for SNP detection is also provided by Fei and Smith (Fei Z. and Smith, L.M. (2000) Rapid Comm Mass Spectrom 14 (11):950-959). Multiplex chip or flow cytometry systems for parallel genotyping may also be utilized, as described by Affymetrix and Axys Pharmaceuticals (Fan, J.B. et al

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(2000)Genome Res 10(6):853-860; Armstrong, B. et al (2000)  
Cytometry 40(2):102-108).

Please replace the paragraph beginning at page 37, line 27,  
with the following:

In a further aspect, the present invention provides a  
diagnostic assay for determining cytochrome P450 3A5 (CYP3A5)  
intron 3 genotype of a subject which comprises  
(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5)PCR fragment from  
said nucleic acid using a set of primers, wherein said set of  
primers contains primer X and primer Y; wherein

(i) the X primer is complementary to a region 5' to the  
point mutation site at nucleotide 22,893 of GENBANK sequence  
accession no. AC005020 (also depicted herein as nucleotide 23 of  
SEQ ID NO: 73 ~~of Figure 3~~);

(ii) the Y primer is complementary to a region 3' to the  
point mutation site at nucleotide 22,893 of GENBANK sequence  
accession no. AC005020 (also depicted herein as nucleotide 23 of  
SEQ ID NO: 73 ~~of Figure 3~~);

(c) amplifying the sequence in between primers X and Y, thereby  
obtaining an amplified fragment; and

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(d) sequencing the amplified fragment obtained in step (c),  
thereby determining the cytochrome P450 3A5 (CYP3A5) intron 3  
genotype of said subject.

Please replace the paragraph beginning at page 38, line 22,  
with the following:

The present invention also provides and contemplates a  
diagnostic assay for determining cytochrome P450 3A5 (CYP3A5)  
intron 3 genotype of a subject which comprises

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from  
said nucleic acid, which includes at least intron 3, thereby  
obtaining an amplified fragment; and
- (c) treating the amplified DNA fragment obtained in step (b) with  
restriction enzyme in its corresponding restriction buffer to  
detect presence or absence of a point mutation at nucleotide  
22,893 of GENBANK sequence accession no. AC005020 (also depicted  
herein as nucleotide 23 of SEQ ID NO: 73 ~~of Figure 3~~); thereby  
determining the cytochrome P450 3A5 (CYP3A5) genotype of said  
subject.

Please replace the paragraph beginning at page 39, line 3,  
with the following:

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In a particular embodiment, the present invention includes a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises

- (a) isolating nucleic acid from said subject;
- (b) making a first and a second PCR primer wherein

(i) the first PCR primer is complementary to intron 3 and introduces a base change in the PCR product adjacent to or near the point mutation at nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~), such that a restriction site is generated in the presence of a particular nucleotide at nucleotide 22,893; and

(ii) the second PCR primer is complementary to a region 3' to the intron 3 nucleotide 22,893 to GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~);

(c) amplifying the sequence in between the first and the second primers; thereby obtaining an amplified fragment; and

(d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide 22,893, thereby determining the cytochrome P450 3A5 (CYP3A5)

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genotype of said subject.

Please replace the paragraph beginning at page 40, line 5, with the following:

In a further aspect, the present invention provides a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) intron 3 genotype of a subject which comprises

(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a first set of primers, wherein said first set of primers contains primer X and primer Y; wherein

(i) the X primer is complementary to a region 5' to the point mutation site at nucleotide 22,893 of GENBANK accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~);

(ii) the Y primer is complementary to a region 3' to the point mutation nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~);

(c) amplifying the sequence in between primers X and Y, thereby obtaining an first round amplified fragment;

(d) amplifying the first round amplified fragment using a second

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set of primers, wherein said second set of primers contains primer Z and primer W, wherein

(i) primer Z is complementary to intron 3 and introduces a base change in the PCR product adjacent to or near the point mutation at nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~), such that a restriction site is generated in the presence of a particular mutation at nucleotide 22,893; and

(ii) primer W is complementary to a region 3' to intron 3;  
(e) amplifying the sequence in between primers Z and W, thereby obtaining an amplified fragment; and  
(f) treating the amplified fragment obtained in step (e) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide 22,893 thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Please replace the paragraph beginning on page 41, line 11, with the following:

In a particular aspect, the invention provides a method for detecting the presence or activity of cytochrome P450 3A5 (CYP3A5), wherein said cytochrome P450 3A5 (CYP3A5) is measured by:

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(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid, which includes at least intron 3, thereby obtaining an amplified fragment; and  
(c) sequencing the amplified fragment obtained in step (b), thereby determining the cytochrome P450 3A5 (CYP3A5) intron 3 genotype of said subject; wherein the detection of an A nucleotide at nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~); indicates the presence or activity of said cytochrome P450 3A5 (CYP3A5) in said sample.

Please replace the paragraph beginning at page 42, line 9, with the following:

In a further aspect, the present invention provides a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) exon 7 genotype of a subject which comprises

(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a set of primers, wherein said set of primers contains primer X and primer Y; wherein

(i) the X primer is complementary to a region 5' to the

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point mutation site at nucleotide 30,597 of GENBANK accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~);

(ii) the Y primer is complementary to a region 3' to the point mutation site a nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~);

(c) amplifying the sequence in between primers X and Y, thereby obtaining an amplified fragment: and

(d) sequencing the amplified fragment obtained in step (c), thereby determining the cytochrome P450 3A5 (CYP3A5) exon 7 genotype of said subject.

Please replace the paragraph beginning at page 43, line 4, with the following:

The present invention also provides and contemplates a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises

(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid, which includes at least exon 7, thereby obtaining an amplified fragment; and



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(c) treating the amplified DNA fragment obtained in step (b) with restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~); thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Please replace the paragraph beginning at page 43, line 15, with the following:

In a particular embodiment, the present invention includes a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) genotype of a subject which comprises

- (a) isolating nucleic acid from said subject;
- (b) making a first and a second PCR primer wherein

- (i) the first PCR primer is complementary to exon 7 and introduces a base change in the PCR product adjacent to or near the point mutation at nucleotide 30,597 of GENBANK accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~), such that a restriction site is generated in the presence of a particular nucleotide at nucleotide 30,597; and

- (ii) the second PCR primer is complementary to a region 3' to

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the exon 7 nucleotide 30,597 GENBANK accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~);

(c) amplifying the sequence in between the first and the second primers; thereby obtaining an amplified fragment; and

(d) treating the amplified fragment obtained in step (c) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide 30,597, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Please replace the paragraph beginning at page 44, line 18, with the following:

In a further aspect, the present invention provides a diagnostic assay for determining cytochrome P450 3A5 (CYP3A5) exon 7 genotype of a subject which comprises

(a) isolating nucleic acid from said subject;  
(b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid using a first set of primers, wherein said first set of primers contains primer X and primer Y; wherein

(i) the X primer is complementary to a region 5' to the point mutation site at nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of

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SEQ ID NO:74 ~~of Figure 5~~);

(ii) the Y primer is complementary to a region 3' to the point mutation site at nucleotide 30,597 of GENBANK accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~);

(c) amplifying the sequence in between primers X and Y, thereby obtaining ~~an~~ a first round amplified fragment;

(d) amplifying the first round amplified fragment using a second set of primers, wherein said second set of primers contains primer Z and primer W, wherein

(i) primer Z is complementary to exon 7 and introduces a base change in the PCR product adjacent to or near the point mutation at nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted herein as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~), such that a restriction site is generated in the presence of a particular mutation at nucleotide 30,597; and

(ii) primer W is complementary to a region 3' to exon 7;  
(e) amplifying the sequence in between primers Z and W, thereby obtaining an amplified fragment; and  
(f) treating the amplified fragment obtained in step (e) with a restriction enzyme in its corresponding restriction buffer to detect presence or absence of a point mutation at nucleotide

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30,597, thereby determining the cytochrome P450 3A5 (CYP3A5) genotype of said subject.

Please replace the paragraph beginning at page 45, line 25, with the following:

In a particular aspect, the invention provides a method for detecting the presence or activity of cytochrome P450 3A5 (CYP3A5), wherein said cytochrome P450 3A5 (CYP3A5) is measured by:

- (a) isolating nucleic acid from said subject;
- (b) amplifying a cytochrome P450 3A5 (CYP3A5) PCR fragment from said nucleic acid, which includes at least exon 7, thereby obtaining an amplified fragment; and
- (c) sequencing the amplified fragment obtained in step (b), thereby determining the cytochrome P450 3A5 (CYP3A5) exon 7 genotype of said subject; wherein the detection of a G nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) indicates the presence or activity of said cytochrome P450 3A5 (CYP3A5) in said sample.

Please replace the paragraph beginning at page 48, line 4, with the following:

In a particular embodiment, the first PCR primer in a test kit

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of the present invention introduces a *Tru9I/MseI* restriction site in the presence of an A nucleotide at nucleotide 22,893 of GENBANK sequence accession no. AC005020 (also depicted as nucleotide 23 of SEQ ID NO:73 ~~of Figure 3~~).

Please replace the paragraph beginning at page 49, line 1, with the following:

In a further particular aspect, the present invention provides a test kit for determining cytochrome P450 3A5 (CYP3A5) genotype in a eukaryotic cellular sample, comprising:

- (a) a predetermined amount of a first PCR primer complementary to nucleotide 30,597 region of GENBANK sequence accession no. AC005020 (also depicted as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~) that introduces a base change in the PCR product adjacent to or near the point mutation at nucleotide 30,597, such that a restriction site is generated in the presence of a particular mutation at nucleotide 30,597 (also depicted as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~);
- (b) a predetermined amount of a second PCR primer complementary to a region 3' to exon 7;
- (c) other reagents; and
- (d) directions for use of said kit.

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Please replace the paragraph beginning at page 49, line 15, with the following:

In a particular embodiment, the first PCR primer in a test kit of the present invention introduces a *Tru9I*/*MseI* restriction site in the presence of an A nucleotide at nucleotide 30,597 of GENBANK sequence accession no. AC005020 (also depicted as nucleotide 29 of SEQ ID NO:74 ~~of Figure 5~~).

Please replace the paragraph beginning at page 78, line 6, with the following:

We performed quantitative immunoblotting of CYP3A5 and CYP3A4 content of tissue preparations as described<sup>14</sup>; purified cDNA-expressed CYP3A5 (a gift from R. Peter) and CYP3A4 purified from human liver were the reference standards<sup>11</sup>. Liver microsomes (10-20  $\mu$ g) and intestinal homogenates (50  $\mu$ g), both of which had been prepared from organ donor tissue<sup>14</sup>, were resolved by electrophoresis, and CYP3A5 was detected with anti-CYP3A5 antibody (Gentest) or anti-CYP3A4 antibody<sup>14</sup>. We determined the integrated optical density of each band by using a Bio-Rad ChemiDoc and ~~QUALITY ONE~~ QUANTITY ONE program.